

## To Cite:

Banu TN, Mandal S. Combined antibacterial activity of *Punica granatum* fruit peel extract with some antibiotics against human pathogenic bacteria: *in vitro* analysis. *Discovery*, 2022, 58(322), 1079-1083

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## Peer-Review History

Received: 06 August 2022

Reviewed & Revised: 11/August/2022 to 12/September/2022

Accepted: 15 September 2022

Published: October 2022

## Peer-Review Model

External peer-review was done through double-blind method.



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DISCOVERY  
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# Combined antibacterial activity of *Punica granatum* fruit peel extract with some antibiotics against human pathogenic bacteria: *in vitro* analysis

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## ABSTRACT

The current study determines the antibacterial activity of pomegranate (*Punica granatum*) fruit peel ethanolic extract (PRE) against gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*) and gram-positive (*Enterococcus faecalis* and *Staphylococcus aureus*) pathogenic bacteria. The PRE showed excellent antibacterial activity against all the test bacteria, and this agent (PRE) in combination with antibiotics (colistin, ciprofloxacin, imipenem and meropenem) had mixed interactions: synergistic and additive for most of the combination, and antagonistic for one combination. Thus, the pomegranate (*Punica granatum*) fruit peel ethanolic extract might be utilized alone or in combination with antibiotics in the treatment of bacterial infection to humans.

**Keywords:** *Punica granatum*, Antibacterial activity, Growth inhibitory index, Zone diameter of inhibition.

## 1. INTRODUCTION

Due to indiscriminate use of antibiotics, the antibiotics capacity of action has been reduced and the bacterial strains become antibiotic resistant (Mandal, 2015). For the reason the drugs normally are used against these bacteria are non-effective. There has been report on the emergence of *Escherichia coli*, *Klebsiella* and *Pseudomonas* showing resistance to multiple antibiotics (Duran et al., 2012). In order to combat the bacterial antibiotic resistances, several authors reported the antibacterial efficacy of plant extracts (Jam et al., 2022; Hajifattahi et al., 2016). The pomegranate edible and non-edible parts have been observed as an excellent antibacterial as well as antioxidative agents (Guerrero-Solano et al., 2020). The pomegranate fruit parts: peel, aril, seeds, and juice, have been reported to be rich in various different kinds of bioactive components (Fawole et al., 2012). Besides, plants of medicinal value are known to display very little or no side effects compared to the synthetic antibiotics ((Hajifattahi et al., 2016)). (Guerrero-Solano et al., 2020) investigated antibacterial activity of different plant extracts including *Punica granatum* against food borne spoilage bacteria. Previously the combined

antibacterial activity of plant extracts and antibiotics had been reported by different authors (Sircar and Mandal, 2016)). Therefore, the current study explores the antibacterial activity of *Punica granatum* fruit peel (available in the local niches: West Bengal, India) against gram-positive and gram-negative clinical bacteria.

## 2. MATERIALS AND METHODS

The gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) as well as gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*) clinical bacterial isolates, as maintained in the laboratory cystine tryptone agar (Hi-Media, India) stabs, were utilized in the current study.

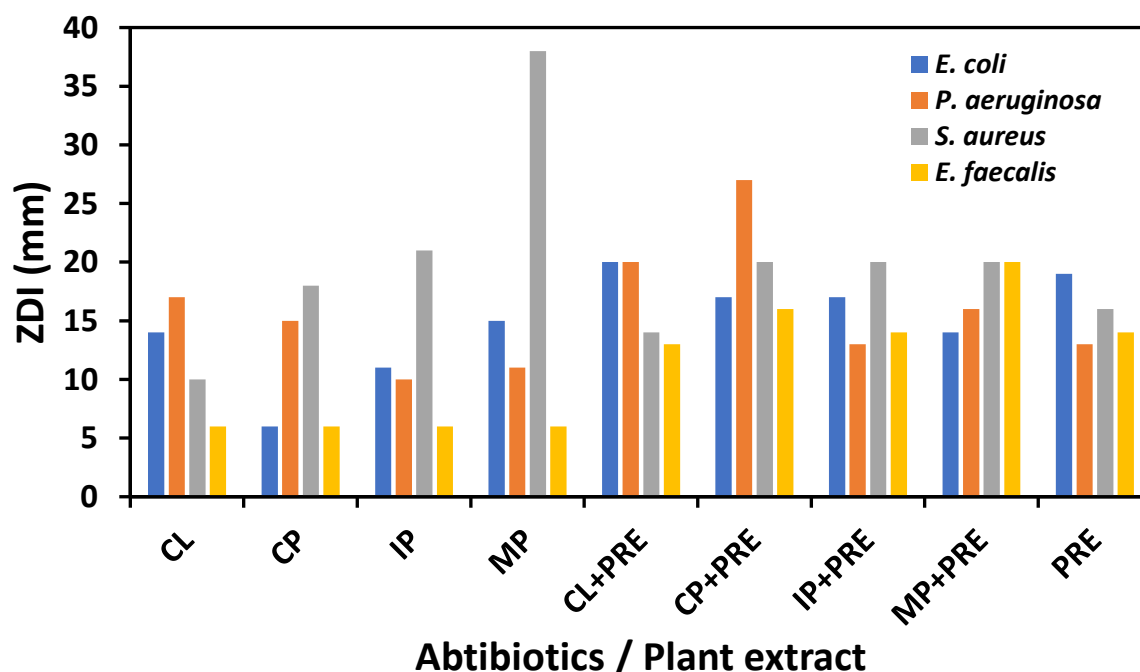
The antibiotic susceptibility testing for the said bacterial isolates was done following disc diffusion (Bauer et al., 1966), using the antibiotics ((Hi-Media, India)): ciprofloxacin (CP; 10 µg/disc), imipenem (IP; 10 µg/disc), colistin (CL; 10 µg/disc) and meropenem (MRP; 10 µg/disc).

The fruit peels from indigenous variety of pomegranate, *Punica granatum* (family: Punicaceae), were collected, dried and grinded for extract preparation using ethanol (Banu and Mandal, 2019). The pomegranate fruit peel ethanolic extract (PRE) was stored at 4 °C for further testing. The antibacterial activity of PRE was determined by disc diffusion (Mandal et al., 2007), following the protocol as explain earlier (Das and Mandal, 2016). ZDI (zone diameter of inhibition) value of  $\geq 7$  mm was accounted sensitivity for PRE to the bacterial isolates (Nascimento et al., 2000).

For combined antibacterial activity of antibiotics (CL, CP, IP and MP) and plant extract (PRE: using 2 mg in 40 µl), on Muller-Hinton agar plates, was determined following the method described earlier (Sircar and Mandal, 2016)). The growth inhibitory index (GII) values from the combined action of antibiotics and plant extracts (PRE) were determined, and the interactions were interpreted as synergistic, additive/indifference or antagonistic with GIIs  $>0.5$ , 0.5 and  $<0.5$ , respectively, as reported by Mandal et al. (2010).

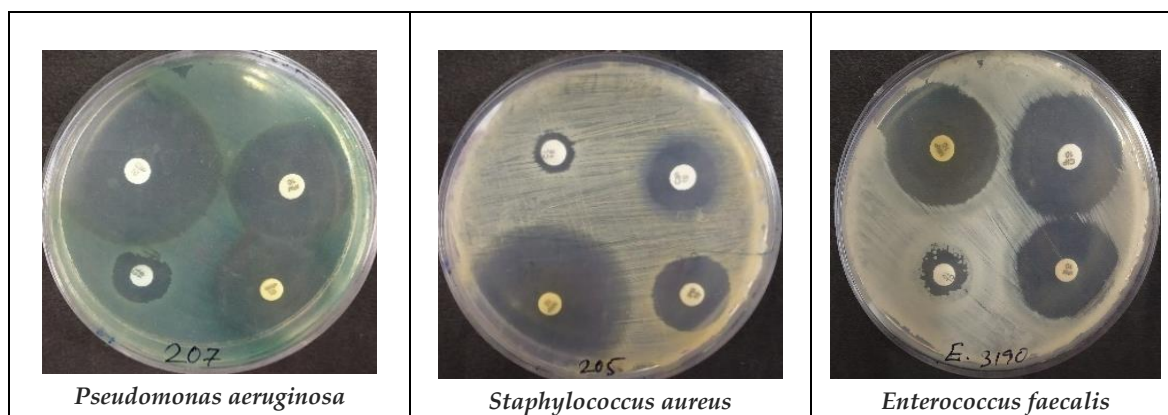
## 3. RESULTS AND DISCUSSION

Various phytochemicals remain the excellent alternatives to synthetic antibiotics in preventing bacterial antibiotic resistance (Mandal et al., 2007). The antibacterial activity of four antibiotics and the plant PRE, alone and in combination, against gram-negative and gram-positive clinical bacteria are represented in Figure 1. Among the test bacterial isolates, *Enterococcus faecalis* showed resistance (ZDI: 6 mm) to all the antibiotic tested. *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* had ZDIs of 10 – 17, 6 – 18, 10 – 21 and 11 – 38 mm against CL, CP, IP and MP, respectively (Figure 1).

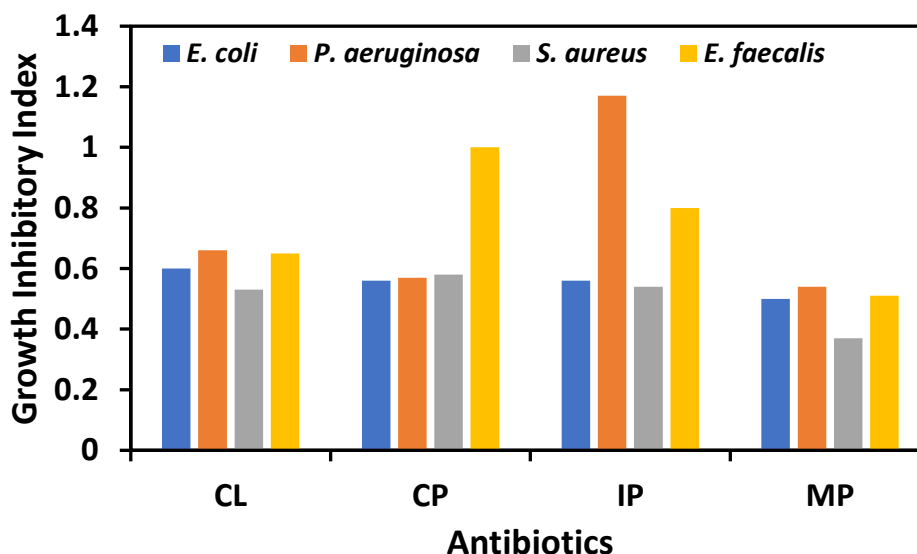


**Figure 1:** Antibacterial activity of *Punica granatum* fruit peel ethanolic activity alone and in combination with antibiotics. CL: colistin; CP: ciprofloxacin; IP: imipenem; MP: meropenem; PRE: pomegranate fruit peel ethanolic extract.

The plant extract (PRE) had excellent antibacterial activity against *Escherichia coli* (ZDI: 19 mm), *Pseudomonas aeruginosa* (ZDI: 13 mm), *Staphylococcus aureus* (ZDI: 16 mm) and *Enterococcus faecalis* (ZDI: 14 mm) by disc diffusion. The highest and lowest growth inhibitory activity had been displayed by hydroalcoholic extract of *Punica granatum* petal against *Streptococcus sanguinis* (ZDI: 22 mm) and *Enterococcus faecalis* (ZDI: 16 mm) (Hajifattahi et al., 2016). Among the ethanolic extracts of various parts of pomegranate pomegranate the fruit peel extract showed strong inhibitory activity against test bacteria (Dahham et al., 2010). Herein, the antibiotics: CL, CP, IP and MP, when tested in combination with the plant extract (PRE) against the test pathogenic bacteria, displayed ZDIs 13 – 20, 16 – 27, 13 – 20 and 14 – 20 mm respectively (Figure 1). The information as depicted in Figure 2 indicate the combined antibacterial activity of PRE and antibiotics.



**Figure 2:** The *Punica granatum* antibacterial activity test results



**Figure 3:** GIIs (growth inhibitory indices) in combined activity of *Punica granatum* fruit peel ethanolic activity and antibiotics against pathogenic bacteria. CL: colistin; CP: ciprofloxacin; IP: imipenem; MP: meropenem

In our earlier report the synergistic interaction between antibiotics and plant extracts have reported (Das and Mandal, 2022). The GII for the test bacterial isolates: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis* are represented in Figure 3, combined with PRE, the antibiotics: CL, CP and IP had GIIs of 0.53 – 0.66, 0.56 – 1.0 and 0.54 – 1.17. The synergistic activity was seen except *S. hominis* against in combined treatment; synergistic (GIIs: 0.51-0.89), antagonistic (GIIs: 0.37-0.47), respectively, and thus the interactions were synergistic against all the bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*). Antibiotic MP combined with PRE, however, showed mixed interactions: synergistic

(for *Pseudomonas aeruginosa* and *Enterococcus faecalis* having GIIs >0.5), additive or indifference (for *Escherichia coli* having GIIs: 0.5) and antagonistic (for *Staphylococcus aureus* having GIIs <0.5). The synergistic antibacterial activity between antibiotics and plant extracts has been an effective tool to reduce the dosages antibiotics thereby decreasing the unwanted side effects of the antibiotics along with the suppression of emergence of antibiotic resistance among pathogenic bacteria.

#### 4. CONCLUSION

The current study explains the usefulness of application of *Punica granatum* fruit peel alone or in combination of antibiotics in combating bacterial infection caused by gram-negative as well as gram-positive pathogenic bacteria. Also, *Punica granatum* might be a good source of preparation of treatment regimen alternative to synthetic antibiotics. Further studies are, however, required to determine the safety profile and pharmacokinetics of the antibacterial active molecules contained in different of the plant (*Punica granatum*).

#### Funding

This study has not received any external funding.

#### Conflicts of interests

The authors declare that there are no conflicts of interests.

#### Data and materials availability

All data associated with this study are present in the paper.

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